

**Genomic analysis of multi-drug resistant porcine commensal  
*Escherichia coli* sourced from a commercial production  
operation in Australia**

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## **Certificate of authorship**

I, Tiziana Maria Vittoria Zingali declare that this thesis is submitted in fulfilment of the requirements for the award of Doctor of Philosophy, in the faculty of Science, School of Life Sciences at the University of Technology Sydney.

This thesis is wholly my own work unless otherwise reference or acknowledged. In addition, I certify that all information sources and literature used are indicated in the thesis.

This document has not been submitted for qualifications at any other academic institution. This research is supported by an Australian Government Research Training Program.

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## **Statement**

This thesis is by compilation. Data Chapter 5 has been published and listed below. Data chapters 4 and 6 are in publication style manuscript that have been prepared for journal submission. Some figures and tables lost their original resolution due to the margins required by thesis formatting. Please refer to figures attached in PDF format.

## List of publications

*Paper 1, Chapter 5*

### **Diversity of P1 phage-like elements in multidrug resistant *Escherichia coli***

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*Genomic analysis of multidrug-resistant commensal Escherichia coli from healthy Australian swine.*

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## Table of Contents

CERTIFICATE OF AUTHORSHIP .....	I
ACKNOWLEDGEMENTS .....	II
STATEMENT .....	IV
LIST OF PUBLICATIONS .....	V
ABBREVIATIONS .....	X
ABSTRACT .....	XI
<b>1 CHAPTER 1: THESIS OVERVIEW.....</b>	<b>1</b>
1.1 OVERVIEW .....	1
1.2 AIMS .....	1
1.3 KNOWLEDGE ADDED TO THE FIELD .....	1
<b>2 CHAPTER 2: LITERATURE REVIEW.....</b>	<b>3</b>
2.1 ANTIMICROBIAL RESISTANCE: ONE OF THE BIGGEST CONTEMPORARY CHALLENGES FOR HUMANITY .....	3
2.2 <i>ESCHERICHIA COLI</i> : COMMENSAL, HARMFUL PATHOGEN AND ENVIRONMENTAL POLLUTANT .....	4
2.3 THE RISE OF ANTIMICROBIAL RESISTANCE IN LIVESTOCK .....	5
2.3.1 <i>MDR E. coli in swine production practices</i> .....	6
2.3.2 <i>How swine manure contributes to the spread of AMR</i> .....	8
2.4 MOBILE GENETIC ELEMENTS: THE KEY TO UNDERSTAND THE EVOLUTION OF ANTIMICROBIAL RESISTANCE .....	9
2.4.1 <i>Plasmids</i> .....	10
2.4.2 <i>Class 1 integrons</i> .....	11
2.4.3 <i>Transposons</i> .....	12
2.4.4 <i>Insertion sequences</i> .....	13
2.4.5 <i>Phage-like elements</i> .....	14
2.5 <i>MDR E. COLI</i> AND POTENTIAL ZONOSIS: THE BACTERIAL FLOW BETWEEN SOWS, PIGLETS AND HUMANS .....	14
2.6 CONCLUSIONS .....	15
2.7 REFERENCES .....	16
<b>3 CHAPTER 3: OVERVIEW OF THE RESEARCH METHODOLOGY.....</b>	<b>25</b>
3.1 STUDY DESIGN .....	25
3.2 METHODOLOGY .....	25
3.3 DNA ISOLATION .....	26
3.4 POLYMERASE CHAIN REACTION (PCR) .....	26
3.5 DNA SEQUENCING .....	26

3.5.1	<i>Illumina HiSeq</i> .....	26
3.5.2	<i>Pacific Bioscience sequencing platform</i> .....	26
3.5.3	<i>Oxford Nanopore Technologies MinION</i> .....	27
3.6	DNA ASSEMBLY .....	27
3.6.1	<i>Short reads assembly</i> .....	27
3.6.2	<i>Hybrid assembly</i> .....	27
3.7	<i>E. COLI CHARACTERISATION AND PHYLOGENETIC ANALYSIS</i> .....	27
3.7.1	<i>Multilocus sequence typing (MLST)</i> .....	27
3.7.2	<i>Phylosift</i> .....	28
3.8	GENE SCREENING .....	28
3.8.1	<i>Basic Local Alignment Search Tool (BLAST)</i> .....	28
3.8.2	<i>Gene databases</i> .....	28
3.9	GENOME ANNOTATION.....	28
3.10	COMPARATIVE ANALYSIS.....	29
3.10.1	<i>BLAST Ring Image Generator (BRIG)</i> .....	29
3.10.2	<i>Easyfig</i> .....	29
3.11	DATA VISUALISATION .....	29
3.11.1	<i>Snapgene (GSL Biotech)</i> .....	29
3.11.2	<i>Interactive tree of life (iTOL)</i> .....	29
3.12	REFERENCES .....	30
<b>4</b>	<b>CHAPTER 4: WHOLE GENOME SEQUENCING ANALYSIS OF PORCINE FAECAL COMMENSAL <i>ESCHERICHIA COLI</i> CARRYING CLASS 1 INTEGRONS FROM SOWS AND THEIR OFFSPRING .....</b>	<b>32</b>
4.1	DECLARATION.....	32
4.2	ABSTRACT.....	32
4.3	IMPACT STATEMENT .....	33
4.4	DATA SUMMARY .....	33
4.5	INTRODUCTION.....	34
4.6	METHODS.....	35
4.6.1	<i>Field animal trial</i> .....	35
4.6.2	<i>Faecal samples collection, E. coli isolation and identification</i> .....	36
4.6.3	<i>Isolate identifiers</i> .....	36
4.6.4	<i>DNA extraction and whole genome sequencing</i> .....	37
4.6.5	<i>Assembly statistics</i> .....	37
4.6.6	<i>Gene identification, serotyping, phylogrouping, phylogenetic analysis and multilocus sequence typing (MLST)</i> .....	37
4.7	RESULTS.....	38
4.7.1	<i>Class 1 integrase gene presence</i> .....	38
4.7.2	<i>Phylogroups, sequence types and serotypes</i> .....	38
4.7.3	<i>Phylogenetic analysis</i> .....	39



4.7.4	<i>Antimicrobial resistance genes (ARGs)</i> .....	39
4.7.5	<i>Class 1 integron structures</i> .....	41
4.7.6	<i>Virulence associated genes (VAGs)</i> .....	42
4.7.7	<i>Plasmid incompatibility groups</i> .....	42
4.8	DISCUSSION.....	43
4.9	REFERENCES .....	48
4.10	DATA BIBLIOGRAPHY .....	54
4.11	FIGURES .....	56
	<i>Figure 4.1:</i> .....	57
	<i>Figure 4.2:</i> .....	58
	<i>Figure 4.3:</i> .....	59
<b>5</b>	<b>CHAPTER 5: DIVERSITY OF P1 PHAGE-LIKE ELEMENTS IN MULTIDRUG RESISTANT <i>ESCHERICHIA COLI</i></b> .....	<b>60</b>
5.1	DECLARATION.....	60
5.2	ABSTRACT.....	62
5.3	INTRODUCTION.....	62
5.4	METHODS.....	64
5.4.1	<i>Bacterial strains</i> .....	64
5.4.2	<i>PacBio sequencing, annotation and bioinformatic analysis of MGEs genomes</i> .....	64
5.4.3	<i>Phage induction and lysogenization of commensal <i>E. coli</i></i> .....	65
5.4.4	<i>Characterization of lysogenic <i>E. coli</i></i> .....	65
5.4.5	<i>Conjugation assay</i> .....	66
5.4.6	<i>Occurrence of phage-like plasmids in Australian <i>E. coli</i> collections and NCBI databases</i> 66	
5.5	RESULTS.....	67
5.5.1	<i>Genomes of P1-like plasmids</i> .....	67
5.5.2	<i>Common regions of difference (RD)</i> .....	67
5.5.3	<i>Unique features of P1-like plasmids</i> .....	68
5.5.4	<i>pTZ20_1P self-transfer ability</i> .....	70
5.5.5	<i>Incidence of P1-like plasmids in Australian <i>E. coli</i></i> .....	70
5.6	DISCUSSION.....	71
5.7	REFERENCES .....	74
5.8	FIGURES .....	79
	<i>Figure 5.1</i> .....	79
	<i>Figure 5.1</i> .....	80
	<i>Figure 5.2</i> .....	81
	<i>Figure 5.3</i> .....	83
	<i>Figure 5.3</i> .....	84

<b>6</b>	<b>CHAPTER 6: FIRST GENOMIC CHARACTERISATION OF AN INC<sub>HI2</sub> ST4 PLASMID IN AUSTRALIA .....</b>	<b>87</b>
6.1	DECLARATION.....	87
6.2	ABSTRACT.....	88
6.3	BACKGROUND.....	88
6.4	METHODS.....	90
6.4.1	<i>Strain, DNA isolation and whole genome sequencing .....</i>	<i>90</i>
6.4.2	<i>Gene screening, plasmid typing and alignment analysis .....</i>	<i>91</i>
6.5	RESULTS AND DISCUSSION.....	91
6.5.1	<i>Gene content of pTZ41_1P_HI2.....</i>	<i>91</i>
6.5.2	<i>Phylogeny and comparative genomic analysis of Inc<sub>HI2</sub> ST4 plasmids in Genbank database .....</i>	<i>95</i>
6.5.3	<i>Comparative analysis of sul3 integrons in pTZ41_1P_HI2 and pSDE-SvHI2 .....</i>	<i>96</i>
6.6	CONCLUSION.....	97
6.7	REFERENCES .....	98
6.8	FIGURES .....	105
	<i>Figure 6.1.....</i>	<i>105</i>
	<i>Figure 6.2.....</i>	<i>106</i>
	<i>Figure 6.3.....</i>	<i>107</i>
	<i>Figure 6.4.....</i>	<i>108</i>
<b>7</b>	<b>CHAPTER 7: GENERAL DISCUSSION AND FUTURE DIRECTIONS .....</b>	<b>109</b>
7.1	AIM 1 .....	111
7.2	AIM 2 .....	112
7.3	AIM 3 .....	114
7.4	FUTURE DIRECTIONS .....	116
7.5	REFERENCES .....	118

## Abbreviations

AMR	Antimicrobial Resistance
ARG	Antimicrobial Resistance Gene
VAG	Virulence-Associated Gene
MGE	Mobile Genetic Element
IS	Insertion Sequence
DNA	Deoxyribonucleic Acid
bp	Base Pair
CRL	Complex Resistance Locus
ESBL	Extended-Spectrum Beta-Lactamase
PWD	Post-Weaning Diarrhea
ETEC	Enterotoxigenic <i>Escherichia coli</i>
IPEC	Intestinal Pathogenic <i>Escherichia coli</i>
ExPEC	Extraintestinal Pathogenic <i>Escherichia coli</i>
HGT	Horizontal Gene Transfer
MDR	Multiple-Drug (Antimicrobial) Resistance
MLST	Multiple Locus Sequence Typing
pMLST	Plasmid Multilocus Sequence Typing
ST	Sequence Type
WGS	Whole Genome Sequencing

## Abstract

The swine industry represents one of the biggest food animal industries worldwide, with approximately 1 billion animals slaughtered each year. Antimicrobials such as antibiotics and heavy-metals are used to maintain the health of intensively reared swine, preventing and containing the spread of diseases and the consequent economical loss for the industry. However, reliance on antimicrobials drives the development of antimicrobial resistance (AMR) in the gastrointestinal flora, particularly in *Escherichia coli*, one of the most important commensal microorganisms of the gut microflora of vertebrates. As a consequence of the antimicrobial selective pressure, porcine faecal material contains a heavy burden of resistant bacteria carrying antimicrobial resistance genes (ARGs), which are captured and mobilised by mobile genetic elements (MGEs) that are released in the environment and act as pollutants.

The aim of this research was conducting whole genome sequencing (WGS) analysis of Australian porcine commensal *E. coli* to create baseline knowledge on their genomic background and on the presence of AMR. This thesis provides insights into the carriage of class 1 integrons and plasmids as vectors of ARGs and virulence associated genes (VAGs), and how those genes are captured and assembled in complex resistance gene loci (CRL). This thesis also investigated the role played by plasmids, transposons and insertion elements in mobilising CRL. The presence of unique genomic signatures useful to track multiple-drug resistant (MDR) *E. coli* in different settings, was identified.

A collection of 117 MDR porcine commensal *E. coli* was characterised using short read WGS technology. *E. coli* was sourced from sows and their offspring, with no history of previous antimicrobial treatments. *E. coli* belonging to phylogroup A and B1 predominated. Forty-five Sequence Types (STs) were identified, with prevalence of ST10 and ST20. Resistance to clinically-important antimicrobial agents was not observed. ARGs were mostly associated with atypical class 1 integrons, whose 3'-CS was modified by the presence of IS26.

Long read sequencing technologies enabled assembly of complete plasmid sequences, and improved the resolution of large multiple-drug resistance regions carried by IncHI2

plasmids and phage-like plasmids, highlighting their role in mobilising resistance determinants.

To date, genomic studies primarily report the presence of ARGs and VAGs in well-known pathogenic porcine *E. coli* lineages, which may represent a bias in genomic data available in public repositories. The present thesis will play a role in filling this knowledge gap by providing WGS analysis of commensal *E. coli* in healthy Australian swine. Although the size of the study collection is relatively small, this thesis identified a wide variety of STs, ARGs and VAGs, and identified the dominant *E. coli* lineages that preferentially colonise the porcine lower gastrointestinal tract.